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(demembranated or membrane adj disrupted) and sperm	10

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(demembranated or membrane adj
disrupted) and sperm

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USPT	(demembranated or membrane adj disrupted) and sperm	10	<u>L8</u>
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USPT	gene adj transger and sperm	0	<u>L6</u>
USPT	gene and sperm	4398	<u>L5</u>
USPT	transfer and sperm	3008	<u>L4</u>
USPT	transgenic and sperm adj head	5	<u>L3</u>
USPT	transgenic and sperm	1147	<u>L2</u>
USPT	transgenic	5046	<u>L1</u>

L23 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2000 ACS
 ACCESSION NUMBER: 2000:133382 CAPLUS
 TITLE: Method of performing transgenesis
 INVENTOR(S): Perry, Anthony C. F.; Wakayama, Teruhiko
 PATENT ASSIGNEE(S): USA
 SOURCE: PCT Int. Appl., 45 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2000008924	A1	20000224	WO 1999-US18429	19990811
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
PRIORITY APPLN. INFO.:			US 1998-96078	19980811
			US 1999-134251	19990513

AB The invention provides a method for generating transgenic animals and cells by the coinserction of nucleic acid and a nucleus into an unfertilized oocyte. Preferably, the coinserction is by **microinjection** and more preferably by piezo-electrically actuated **microinjection**. Transgene (tg) expressing embryos are here produced following coinjection of unfertilized mouse oocytes with **sperm heads** and exogenous DNA encoding either a green fluorescent protein (GFP) or .beta.-galactosidase reporter. The microinjected oocyte may be allowed to develop into differentiated cells or stem cells; into an embryo in vitro prior to transfer into a host surrogate mother; or it may be transferred directly into a host surrogate mother. Embryonic development can occur to term, such that the offspring possess transgenic modifications that may alter their characteristics (phenotype) and are, in turn, transmitted to their offspring.

L7 ANSWER 4 OF 10 MEDLINE
ACCESSION NUMBER: 1998132258 MEDLINE
DOCUMENT NUMBER: 98132258
TITLE: Behavior of transgenic mouse spermatozoa with galline
protamine.
AUTHOR: Maleszewski M; Kuretake S; Evenson D; Yanagimachi H;
Bjordahl J; Yanagimachi R
CORPORATE SOURCE: Department of Anatomy and Reproductive Biology, University
of Hawaii, Honolulu 96822, USA.
CONTRACT NUMBER: HD-03402 (NICHD)
SOURCE: BIOLOGY OF REPRODUCTION, (1998 Jan) 58 (1) 8-14.
Journal code: A3W. ISSN: 0006-3363.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY WEEK: 19980502

AB General morphology, physical and chemical stability of nuclei, and
postfertilization behavior of spermatozoa from transgenic mice [TgN (Prml
Gal) 223 Bri] containing nuclear **avian** protamine (galline) were
compared to those in the spermatozoa of wild-type (Wild) mice. Galline to
protamine I ratios in spermatozoal nuclei of transgenic mice, strains

3175 (T75) and 3177 (T77), were 1.94 and 5.62, respectively. Live T75 and T77
spermatozoa were indistinguishable in their gross morphology from Wild
spermatozoa. However, unlike Wild and T75 spermatozoa, T77 spermatozoa
were vulnerable to mechanical handling, as about 40% of heads and tails
were separated after gentle pipetting in suspension. Motility of T77
spermatozoa was markedly inferior to that of T75 and Wild. Chromatin
heterogeneity and instability of transgenic spermatozoal nuclei were
evident by transmission electron microscopy, staining reaction to Giemsa,
and, as apparent by both light microscopy and flow cytometry, reaction to
SDS detergent. Wild and T75 spermatozoa fertilized 90% and 60% of
zona-intact **oocytes** in vitro, respectively. T77 spermatozoa
completely failed to fertilize and bound to zona surfaces very weakly,

and
none of them inserted their heads into the zona. Although inefficiently,
T77 spermatozoa could fertilize zona-free **oocytes** in vitro,
indicating some ability to undergo capacitation and spontaneous acrosome
reaction in vitro. After microsurgical injection into **oocytes**,
the rate of nuclear decondensation was the greatest in rooster
spermatozoa, followed by T77, T75, and Wild spermatozoa.

L9 ANSWER 6 OF 12 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 96168878 EMBASE

DOCUMENT NUMBER: 1996168878

TITLE: **Culture** of naked quail (*Coturnix coturnix japonica*) ova in vitro for **avian** transgenesis: **Culture** from the single-cell stage to hatching with pH-adjusted chicken thick albumen.

AUTHOR: Ono T.; Murakami T.; Tanabe Y.; Mizutani M.; Mochii M.; Eguchi G.

CORPORATE SOURCE: Laboratory of Developmental Biology, Faculty of Agriculture, Shinshu University, Ina 399-45, Japan

SOURCE: Comparative Biochemistry and Physiology - A Physiology, (1996) 113/3 (287-292).

ISSN: 0300-9629 CODEN: CBPAB5

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 021 Developmental Biology and Teratology
029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We first examined the pH change of the albumen of quail (*Coturnix coturnix*

japonica) eggs before and after they were laid, as well as that of laid eggs. The pH rose rapidly after laying and continued to increase gradually

in storage. Incubation at 37.5.degree.C accelerated the increase in the pH

of infertile eggs, while that of fertile eggs remained low during incubation. Referring to these results, we obtained a protocol for producing quail hatchlings by **culture** in vitro from naked ova. The naked ovum was filled with chicken (*Gallus domesticus*) thick albumen, the pH of which had been adjusted to 7.2-7.4. The ovum was cultured at 41.5.degree.C in 20% CO2 in air for the first 24 h. Then the embryo was moved to a surrogate quail egg shell that had been filled with non-pH-adjusted chicken thin albumen and cultured for a further 48 h in 100% air. The embryo was transferred again to a surrogate chicken egg shell and cultured under the same conditions until hatching. The **culture** yielded quail chicks with a hatchability of 19.4%. The method proposed here should be applicable to the production of transgenic birds.

L7 ANSWER 3 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1999:289854 BIOSIS

DOCUMENT NUMBER: PREV199900289854

TITLE: **Fertilization** and early **avian** development.

AUTHOR(S): Stepinska, Urszula (1)

CORPORATE SOURCE: (1) Jastrzebiec, 05-551, Mrokow Poland

SOURCE: Postepy Biologii Komorki, (1999) Vol. 26, No. SUPPL. 12, pp. 73-78.

ISSN: 0324-833X.

DOCUMENT TYPE: Article

LANGUAGE: Polish

SUMMARY LANGUAGE: English; Polish

AB The early developmental stages of the bird are rather poorly understood and are the subject of endless discussion. The reason for this has been the difficulty in obtaining the experimental material - the **fertilization** and early embryogenesis (cleavage and area pellucida formation) take place in the hen's oviduct, besides, only 1 oocyte is ovulated every 24 hr. Birds exhibit physiological polyspermy, i. e. many sperms enter the egg, however only one of them participates in the formation of zygote nucleus, whereas the rest of them degenerate at early cleavage stages. This could suggest the presence of some kind of late block to polyspermy in the cytoplasm of **avian** egg. However, the factors participating in the block are not known. It is suggested that DNase activity present in the germinal discs of quail preovulatory **oocytes**, might be responsible for degradation of supernumerary sperm DNA in the early **avian** embryo.

21 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:343776 BIOSIS
DOCUMENT NUMBER: PREV199800343776
TITLE: Morphology of a sterile, tetraploid, hybrid whiptail lizard
(Squamata: Teiidae: Cnemidophorus.
AUTHOR(S): Hardy, Laurence M. (1); Cole, Charles J.
CORPORATE SOURCE: (1) Dep. Herpetol., American Museum Natural History {a}
Dep. Herpetol., American Museum Natural History USA
SOURCE: American Museum Novitates, (June 10, 1998) Vol. 0, No. 3228, pp. 1-16.
ISSN: 0003-0082.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Experimental hybridization with whiptail lizards has been conducted in order to improve understanding of the evolution of parthenogenesis in vertebrates and the effects of horizontal **gene transfer** in Cnemidophorus, the systematics of which has been confused owing to the reticulate phylogeny within the genus. Here we describe the external morphology and reproductive tissue histology of a sterile tetraploid hybrid between *C. sonorae* (triploid, unisexual) X *C. tigris* (diploid, bisexual), and compare her to her parents and siblings that developed from unfertilized eggs (normally cloned *C. sonorae*). This may help to identify F1 hybrids that are found in nature and may help to determine whether they are sterile without conducting extensive laboratory breeding programs. Considering that the maternal parent (*C. sonorae*) represented a clone that was of hybrid origin itself, the four genomes in the tetraploid hybrid historically were derived from three hybridization events among three bisexual species of Cnemidophorus, probably as follows: ((*inornatus* female X *burti* male) X *burti* male) X *tigris* male. The tetraploid inherited 100% of its mother's genes and morphologically was very similar to her and her cloned offspring, particularly in scalation. Nevertheless, it was slightly larger than its siblings at hatching, grew faster than its siblings, attained a larger size, and, beginning at an age of six months, developed dorsal spots reflecting paternal traits in its color pattern. However, if this lizard had been found in nature, without any knowledge of its life history and in the absence of genetic data, it could easily have been misidentified as *Cnemidophorus exsanguis*, which it resembled more closely than its parental species. Although she reached adult size and lived for more than two years beyond the age at which her cloned siblings produced offspring (nine months), the tetraploid never reproduced. Her ovaries were abnormally small, had poorly defined follicular epithelium with little vascularization, and had either empty or fluid-filled follicles devoid of oocytes. She also had numerous abnormally large mesonephric tubules and few or no cilia in the median oviduct. These traits should be examined in other specimens hypothesized to be sterile F1 hybrid females.